

## Effect of Stevia Leaf Powder on Body Weight – An experimental study

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<sup>1</sup> Conception of study

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<sup>2,3</sup> Manuscript Writing

<sup>5,6</sup> Critical Review

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### Abstract

**Introduction:** The steviol glycosides found in the stevia leaf, possess sweetening as well as antioxidant, anti-microbial, anti-inflammatory, anti hyperglycemic, and anti-hypertensive properties. This study was conducted to determine the effect of Stevia leaf on body weight in obese Sprague Dawley male rats.

**Materials and Methods:** This experimental study was carried out on 90 Sprague Dawley male rats. Group 1 (normal control) was given a standard diet for the entire study period, while the remaining sixty rats were given a high-fat diet (HFD) for 8 weeks to induce obesity. The obese rats were randomly assigned to group 2 (Obese Control) and Group 3 (obese + Stevia) and given HFD for another 6 weeks. The stevia leaf powder was added to the diet of group 3. Diet intake was noted for all the groups. Rats were weighed on the first day of every week, for six weeks, and then at the end of the study period.

**Results:** Body weight of rats in each group, at the start and end of the 6-week intervention period, when compared, showed a significant increase in group 1 ( $p=0.001$ ), and group 2 ( $p = 0.001$ ), while group 3 showed a non-significant increase ( $p = 0.248$ ). The percentage increase in body weight for groups 1, 2, and 3 was 4.8, 9.3, and 1.1, respectively. Graphically, the weekly increase in average body weights of the three experimental groups showed maximum values for group 2.

**Conclusions:** Stevia leaf possesses weight-reducing effects which may be due to inhibition of appetite, reduction in food intake, and consequent weight loss.

**Keywords:** Obesity, weight loss, Non-Nutritive sweeteners, Stevia.

## Introduction

Obesity is a common health issue faced by adults and children alike, in this modern era. In 2016, it was estimated that about 650 million grown-ups and 124 million children and young people were afflicted with this condition, the world over.<sup>1</sup> Interestingly, this is observed in the higher as well as middle and low-income countries, with the latter showing high levels of undernutrition as well, subsequently, resulting in the dual dilemma of malnutrition.<sup>2</sup>

The main concerns associated with obesity include high blood pressure, diabetes mellitus, coronary cardiac disease, and deleterious effects on the reproductive system in both genders.<sup>3</sup> Keeping in view all the associated complications, prevention of obesity has become a major public health concern in many countries.<sup>4</sup>

In order to prevent obesity and its related complications, individuals' recourse to the use of non-nutritive sweeteners (NNS) to bring about a reduction in their caloric intake, thus decreasing body weight and lowering the glucose level in blood.<sup>5</sup> A variety of NNS are readily available and can be classified on the basis of their source as artificial and natural.<sup>6</sup>

Artificial NNS are manufactured substances and have an extremely sweet taste with no caloric value. Frequently used artificial NNS include Aspartame, sucralose, neotame, acesulfame, and saccharine to name a few.<sup>7</sup> Nevertheless, their use is believed to be associated with several complications like headache, faintness, gastrointestinal irritation, liver problems, cancer of the bladder, and leukemias.<sup>8</sup>

Natural NNS include Stevia, monk fruit, liquorice root, etc.<sup>9</sup> Stevia Rebaudiana is a perennial shrub, which was first identified scientifically in Paraguay, South America.<sup>10</sup> The non-caloric sweetening effect is due to various steviol glycosides which are present in the stevia leaf.<sup>11</sup> Besides possessing sweetening properties, this shrub is also believed to exert antioxidant, anti-microbial, anti-inflammatory, antihyperglycemic, and anti-hypertensive effects.<sup>12</sup>

This study was conducted to study the effect of Stevia leaf, a naturally occurring NNS, on the body weight of obese Sprague Dawley male rats.

## Materials and Methods

**Setting:** This experimental study was carried out in the Physiology Department of Islamabad Medical and

Dental College (IMDC), Islamabad, and the National Institute of Health (NIH) after approval of synopsis by the Institutional Review Board of IMDC, Islamabad. The duration of our study was six weeks after the development of the obese model, which took eight weeks.

**Animals used:** A total of 90 healthy Sprague Dawley male rats, 5-6 weeks old, with a weight range of 280-320 grams were selected by simple random sampling from the animal house of NIH, where they were bred. The animal house was in accordance with international standards for the breeding and housing of research animals. The rats were housed 3 per cage, the size of which was according to recommended standards (rectangular cage measuring 900cm<sup>2</sup> base and 23cm height).<sup>13</sup> The cages were kept on metallic racks in a well-ventilated room with controlled conditions of temperature (22-24 °C). A 12-hour light and 12-hour dark cycle was maintained in the animal house of NIH, throughout the study period.<sup>14</sup> Rats were given water, ad libitum, through special plastic bottles fitted in an inverted position at the tops of the cages.

Rodent feed was provided by the animal house of NIH. The standard rodent chow (SC) provided 11% of energy from fat, comprising of lard (HF-L), olive oil (HF-O), coconut fat (HF-C), or fish oil (derived from cod liver, HF-F). The High Fat Diet (HFD) was also prepared at NIH, and provided 42% of energy from fat, comprising of lard (HF-L), olive oil (HF-O), coconut fat (HF-C), or fish oil (derived from cod liver, HF-F).<sup>15</sup>

Thirty rats (group1- normal control) were given standard rodent chow for the entire period, while the remaining sixty rats were given a high-fat diet for a period of 8 weeks to induce obesity. After confirmation of obesity by the use of the Lee index ( Lee obesity index = cube root of body weight in grams / naso-anal length in centimeters), according to which rats having a Lee index > 0.30 are considered obese<sup>16</sup>, they were randomly assigned to group 2 (Obese Control) and Group 3 (Stevia intervention) in equal numbers, while the rats which did not become obese were excluded from the study. Both group 2 and group 3 animals were given a high-fat diet for a further period of 6 weeks. Additionally, Stevia leaf powder was added to the diet of group 3. Dried Stevia leaves were obtained from an online organic grocery store and verified by National Agriculture Research Council (NARC), Islamabad. The cleaned and dried leaves were ground to a fine powder, which was then

mixed into the diet,<sup>17, 18</sup> and given to the rats in a dose of 200 mg/kg body weight.<sup>19, 20</sup>

The net daily food consumption of the three experimental rat groups was expressed as grams consumed per rat per day, and all leftover food was excluded from the calculations. All the rats were weighed at the beginning of the intervention period, after the development of the obese model, and then on the first day of every week, at the same time for six weeks, and ultimately at the end of the study period.

**Data analysis procedure**

Data was analysed using SPSS version 22. Descriptive statistics were used to measure and analyse quantitative variables. The results were presented as mean ± standard deviation. Paired sample t-test for comparing weight before and after the intervention period of six weeks in each of the three groups was used. A graph was plotted for representation of the weekly increase in body weight.

A p-value <0.05 was taken as statistically significant.

**Results**

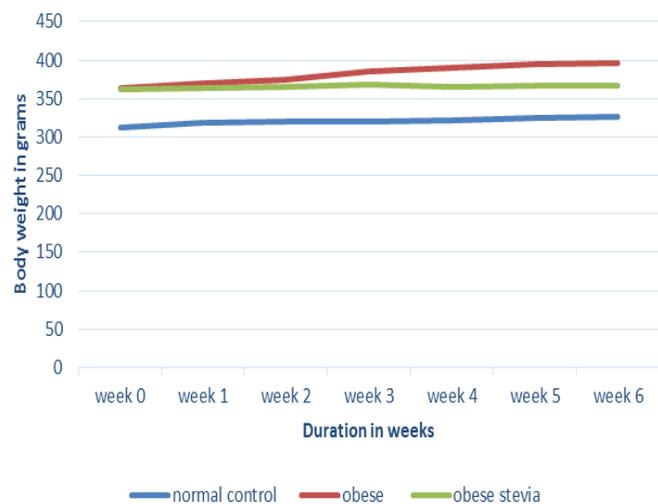
The body weights of rats in each group at the start and at the end of 6-week study period, when compared showed a significant increase in group 1 (p=0.001), and group 2 (p = 0.001), while group 3 showed a non-significant increase (p = 0.248). Moreover, the percentage increase in body weight for the three groups 1, 2, and 3 were 4.8, 9.3, and 1.1 respectively (Table 1). The weekly increase observed in the average body weights of the three experimental groups showed maximum values for the obese group (group 2) with a steeper slope as compared to the other two groups (groups 1 and 3). On calculation of daily feed consumption per rat for group 1 and group 2, an increase from 18.42 ±1.24 grams and 22.50 ± 1.86 grams in the first week to 20.83 ± 1.35 grams and 25.80 ± 1.54 grams respectively, was observed by the end of the sixth week. However, animals of group 3 showed a decrease in food consumption from 22.16 ± 1.02 grams /rat/day to 19.54 ± 1.46 grams.

**Table 1: Comparison of body weight of different experiment groups, at the start and after 6 weeks of intervention, using paired student t-test**

Study Group (n=30 for each group)	Bodyweight in grams			
	At the start of the study (0	At the end of the study (After 6	%age increase in body weight	P-value
Group 1	312.57 ± 5.91	327.14 ± 11.12	4.8%	0.001*
Group 2	363.28 ± 5.64	397.85 ± 8.09	9.3%	0.001*
Group 3	362.71 ± 9.92	366.85 ± 12.94	1.1 %	0.248

	weeks) (mean± SD)	weeks) (mean± SD)		
Group 1	312.57 ± 5.91	327.14 ± 11.12	4.8%	0.001*
Group 2	363.28 ± 5.64	397.85 ± 8.09	9.3%	0.001*
Group 3	362.71 ± 9.92	366.85 ± 12.94	1.1 %	0.248

\* P-value < 0.05 is significant



**Figure 1: Graphic representation of the weekly increase in average body weights of normal control, obese and obese + Stevia groups.**

**Discussion**

Obesity is as a major risk factor, in both genders, for many conditions such as diabetes mellitus, stroke, cardiac disease, and different afflictions of the reproductive system.<sup>21</sup>

Since everyone realizes the importance of maintaining healthy body weight, hence, natural products are being increasingly explored as restorative for preventing the development of metabolic disease. The use of low-calorie sweetening agents is a popular method for helping in weight loss.<sup>22</sup>

Our study based on the effects of NNS on an obese murine model showed that group 3 which was given Stevia, had only a non-significant increase in body weight over a period of 6 weeks, despite administration of the high-fat diet. Our results corroborated with several studies. Elnaga et al (2016) in their study used Stevia extract in different doses (25, 250, 500, and 1000 mg/kg body weight /day) for a

period of 12 weeks, in overweight female rats and found an actual reduction in body weight of their samples with the highest dose group showing the most weight loss.<sup>23</sup> The fact that they observed actual weight loss in their samples as compared to our study in which only slight weight gain was observed despite the continuation of HFD, could be because their intervention period was of 12 weeks as compared to ours which lasted 6 weeks, and the possibility of a gender difference, since they selected female rats for their study.

In another murine study (2018) conducted on hyperlipidemic albino male rats, the use of stevia at different doses for 8 weeks observed that body weight gain was reduced as was the total cholesterol level. Stevia prevented weight gain by lessening caloric utilization, as there was no change in physical activity, during this period. Moreover, it was concluded that stevioside may also cause weight reduction by lowering blood glucose levels and enhancing insulin sensitivity. Stevioside has also been found to reduce fat absorption and enhance fat excretion. Thus, their study demonstrated that the daily consumption of stevia in real-life doses could aid in weight maintenance and the moderation of energy intake.<sup>24</sup>

Similarly, Ahmad et al. (2018) also observed the effect of different doses of stevia in a streptozotocin-induced diabetic rat model, for 8 weeks, and noted a reduction in caloric consumption and decreased body weight and blood glucose.<sup>25</sup>

Nettleton et al (2019) gave young male rats a low dose of Reb A for 9 weeks and observed a change in the composition of the gut microbiota as well as a diminished expression of genes in the nucleus accumbens, which deals with food-seeking behaviour.<sup>26</sup>

## Conclusion

Stevia leaf possesses weight-reducing effects which may be due to suppression of appetite, with a resultant reduction in food intake and consequent weight loss. Stevia is also believed to lessen weight gain by lowering glucose levels, increasing insulin sensitivity, decreasing lipogenic enzyme levels and fat absorption.

## Recommendations

It is recommended that Stevia leaf extract may be used in humans because of its various beneficial properties.

## Materials and Methods

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